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The development of the embryo-sac of Nymphaea advena *

SARA SEATON

(WITH PLATES 18 AND 19)

The taxonomic position of the water-lilies or Nymphaeaceae has always been doubtful because of their peculiar and seemingly inconsistent characteristics. The closed vascular bundles, irregularly placed through the stem, are characteristic of monocotyledons, but the reticulate venation of the large peltate leaves is a dicotyledonous character, while the flowers might belong to either class. Moreover, the fruit presents peculiarities that have been interpreted in various ways. Early investigators studied the seed of nearly or quite mature fruit. Recent investigators, using younger material, have, by their studies of development, made clear points not hitherto known. In 1901, H. L. Lyon declared that "the embryo of *Nelumbo* is genuinely monocotyledonous in its development. The plumule arises laterally and at first there is but one cotyledon which later bifurcates to form the two fleshy bodies." The fact that the radicle does not function is another respect in which *Nelumbo* conforms to well-known monocotyledonous types. Because of the characters of the embryo as well as of the mature plant, Lyon concluded that the Nymphaeaceae should be classified among the monocotyledonous families in a subseries coördinate with the Potamogetonineae, Alismineae, and Butomineae, in the series Helobiae.

In 1902, M. T. Cook followed Lyon with his paper on the embryogeny of *Castalia odorata* and *Nymphaea advena*, in which he confirmed the views already given. In 1904 Schaffner, in his

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paper on morphological peculiarities of Nymphaeaceae and Helobiae, agreed with Lyon and Cook as to the monocotyledonous embryo and vascular bundles, and went farther in saying that he found even the flower could be made to fit into the monocotyledonous scheme. The six sepals of *Nymphaea advena* could be considered a perianth typically trimerous with three sepals and three petals. Even in *Castalia odorata*, said to have four sepals, the sepals are normally three in a cycle, but sometimes there is an expansion of the receptacle causing one sepal of the second cycle to be brought outside. Schaffner maintains that many superficial characteristics of secondary importance, such as similarity to Helobiae in habitat, rhizome habit, leaf forms, and number and arrangement of ovules in ovularies, add strength to the monocotyledonous idea. The embryo of *Castalia odorata* which Conard finds "to have two cotyledons from the first," Schaffner, by dissecting out very young embryos, found to show a resemblance to *Nelumbo* and *Nymphaea* that could much less easily be seen in serial sections. Schaffner concludes his paper with the prediction that with our increasing knowledge of the embryogeny of angiosperms we shall be inclined to divide them into a number of parallel groups rather than maintain the two of our present classification, dicotyledons and monocotyledons. Perhaps a similar idea is expressed in another form by Mottier who calls the Nymphaeaceae anomalous dicotyledons.

Since Cook's paper on embryogeny was based upon the first stages of the development from *Castalia odorata* material, and the later stages from *Nymphaea advena*, I give my own study of the development of the embryo-sac, based altogether on abundant material of *Nymphaea advena* in all the stages.

THE EMBRYO-SAC OF NYMPHAEA ADVENA

The material was collected at Ithaca, N. Y., and Cleveland, Ohio. Twice in July, and again twice in August, for three consecutive summers, buds and flowers of various ages were collected in the bayous of Fall Creek and in the Inlet of Cayuga Lake at Ithaca. In September, at the same place, several plants were uprooted and from the crown small young buds, formed for the next season, were obtained. As these proved to be too young to

show the floral parts, no further material was gathered in the autumn, but collections were made for two consecutive seasons in May, from a small lake thirty miles east of Cleveland. From every collection made, buds and open flowers of varying size and general appearance were selected. Although floating buds of any given collection showed a wide range of gross development, very little variation in the condition of the embryo-sac was found in such material; that is, size and general external appearance are no definite guide to internal development in this species. The season, rather than the gross appearance, is the best criterion of the conditions of development.

Material collected in July and August was carried to the laboratory for killing and fixing; that gathered in May was put in the fixing fluid at the place of collection. At first, the outer floral parts were removed and the pistil cut vertically into from eight to twelve radial pieces, but later all except the youngest ovules were removed from the pistil and fixed separately. In removing the ovules, the large quantity of gelatinous substance made the work difficult. Flemming's chromo-aceto-osmic mixture and chromo-acetic acid were used. Because of the gelatinous substance surrounding the ovules, Wager's alcoholic fixer was tried, but the aqueous fixers gave much better results. The separate ovules, when of small size, were slightly stained *in toto*, with picro-carmine after fixing, in order that they might be more easily seen during subsequent treatment in grades of alcohol, cedar oil, and in paraffin. Sections of varying thickness were cut and stained with different stains, Flemming's triple stain giving the best results. A large number of slides was prepared and all the points figured were observed in many preparations.

This investigation was carried on in the botanical laboratory of Cornell University, under the direction of Professor Atkinson and with the assistance, at first, of Dr. Margaret C. Ferguson and later of Dr. E. J. Durand.

In May, before the integuments begin to develop, a single hypodermal archesporial cell can be distinguished (*figure 1*). This hypodermal cell divides by a transverse wall into an upper cell, the primary parietal cell, and a lower one, the megaspore mother-cell (*figures 2, 3*). The primary parietal cell divides irreg-

ularly, so that the megaspore mother-cell is soon seen to be buried four cells deep (*figure 6*). It expands somewhat and is marked by an abundance of cytoplasm (*figure 5*). At the time of the division of the hypodermal cell, the beginning integuments can be seen, in section, as rounded protuberances from the base of the ovule (*figure 2*). The megaspore mother-cell soon divides transversely into two cells. The succeeding divisions are somewhat irregular. Usually the micropylar daughter-cell next undergoes division (*figure 7*) and after that the chalazal cell, but sometimes the chalazal cell divides first. At other times after the micropylar cell has divided so that a row of three cells has been formed, the middle cell next divides instead of the lower one. Whatever be the succession of divisions, they result in the normal production of an axial row of four cells, or "megaspores," of which the lower one is functional. I have an abundance of material showing the above steps. By irregular divisions of the parietal tissue and of the epidermal tissue at the tip of the ovule simultaneously with the formation of the four megaspores, the functional "megaspore" or embryo-sac mother-cell is buried to the depth of from six to ten cells below the micropylar end of the ovule. The functionless megaspores then degenerate (*figure 9*) so that the embryo-sac mother-cell lengthens toward the micropyle (*figure 10*).

The embryo-sac mother-cell enlarges in the direction of the longitudinal axis of the ovule in the two-nucleate stage (*figure 11*), and broadens in the four-nucleate stage (*figure 12*). The mature embryo-sac further expands toward the micropyle until it is within eight, six, or even four cells of the micropylar end of the ovule (*figures 17, 18*), but never extends to the superficial row of cells as Cook found in *Castalia odorata*. The nucellar tissue between the upper end of the embryo-sac and the micropyle at this time assumes a characteristic appearance; the cells seem crowded, as they are smaller and more compact than at any previous time. They are also arranged in very regular rows. The cytoplasm is so abundant as to leave no vacuoles in these cells and they appear to be stored with food (*figure 17*). At the time the embryo-sac has reached the eight-nucleate stage it occupies one half the length of the ovule (*figure 13*). The polar nuclei fuse and the antipodals soon disappear. The fusion-nucleus is very large (*figures 15, 17*).

Dense cytoplasm surrounds the egg-apparatus, but it is scanty in the rest of the sac, thin streamers extending from the egg-apparatus to the large endosperm-nucleus and from that to the antipodal end of the sac, often following the general direction of the sides but not touching them.

The early embryo-sac appears elliptical in section. The shape of this part of the sac does not change, but in the eight-nucleate stage, at the antipodal end, there is formed a tube-like elongation, which in time reaches to the chalazal end of the ovule. This basal prolongation is always narrower than the older part at the micropylar end of the sac and seldom has a liberal supply of cytoplasm (*figure 13*). At the juncture of the tube-like lower part with broad elliptical upper part of the embryo-sac is a constriction. It is in this constricted part that the large fusion-nucleus or endosperm-nucleus lies. Although the sac lies straight in the axis of the ovule, occasionally, in sectioning, cells from the surrounding tissue appear to be in the sac. These fragments may have given rise to the idea that a cross-wall appears in the sac at this point at the time of the division of the large endosperm-nucleus, which, as stated, always lies in this constricted part (*figures 13, 16*). In my material, this endosperm has been observed soon after division, but a cross-wall in the embryo-sac between the two nuclei, such as described by Cook for *Castalia odorata*, has not been seen. The endosperm-nucleus lying in the constricted part of the sac then divides into two. The upper endosperm-nucleus later divides to form the endosperm tissue while the lower (antipodal) endosperm-nucleus moves down into the chalazal end of the tube-like portion of the sac, enlarges, and often persists until the embryo is quite advanced (*figure 21*). Soon after fertilization of the egg-nucleus the perisperm shows an accumulation of starch. This food supply is remarkably abundant in all the older ovules studied (*figure 20*).

After fertilization the cytoplasm of the sac always gathers about the fertilized egg in a spherical mass. It is vacuolate, with thread-like dense portions radiating from the nucleus to the surface of the sphere of cytoplasm. The cytoplasm from the pollen-tube is dense and takes the stain deeply (*figure 14*). One synergid persists and is very similar to the fertilized egg in appearance

except that it lacks the surrounding regular-shaped mass of cytoplasm. The endosperm-nucleus in the constriction of the sac sometimes divides at this time but usually later. The lower endosperm-nucleus in the chalazal end of the long tube-like portion of the sac is very large and conspicuous with a reticulated surface and a large dense nucleolus (*figures 14, 19*).

The young embryo is nearly spherical, lying against the wall at the micropylar end of the sac, and is nearly surrounded by endosperm. This endosperm tissue is surrounded by perisperm containing a rich food supply. The endosperm tissue never extends into the tube-like base of the embryo-sac but the nucellus closing in from the sides obliterates it, often leaving only the cavity at the extreme chalazal end containing its persisting nucleus (*figures 20, 21*).

SUMMARY

1. The hypodermal cell can be distinguished before the integuments begin to develop.

2. The integuments begin to develop at the time of the division of the hypodermal cell.

3. By the division of the parietal cell the megaspore mother-cell is buried four cells deep.

4. The order in which the four cells of the axial row arise varies, but the lowest one always functions.

5. By simultaneous division of the epidermal and the parietal tissue the embryo-sac is buried six to ten cells below the micropylar end of the ovule.

6. The functionless megaspores degenerate so that the embryo-sac mother-cell lengthens toward the micropyle.

7. The embryo-sac expands until within eight, six, or even four cells of the micropylar end of the ovule, but never to the superficial row of cells as Cook found to be the case in *Castalia odorata*.

8. The nucellar tissue between the upper end of the sac and the micropyle assumes a characteristic appearance; cells crowded, small, dense, in regular rows and stored with food.

9. The eight-nucleate embryo-sac develops a tube-like prolongation toward the chalazal end of the ovule. This tube is always narrower where it joins the broadly elliptical upper part of the sac than throughout the rest of its extent.

10. The large fusion-nucleus lies in the narrow part of the tube-like prolongation of the embryo-sac.

11. When this fusion-nucleus divides, it does not form a wall across the embryo-sac as Cook found in his material.

12. The scanty cytoplasm of the embryo-sac gathers around the fertilized egg in a characteristic manner.

13. The fusion-nucleus may divide at the time of fertilization of the egg but usually this occurs later.

14. The lower endosperm-nucleus, arising from the division of the fusion-nucleus, travels to the chalazal end of the tube-like part of the sac and persists until the embryo has attained considerable size.

15. The embryo is spherical, lying against the wall, almost surrounded by endosperm, within the perisperm, rich in food.

16. The Nymphaeaceae are monocotyledonous in embryology, vascular tissue, habit, and possibly even in floral arrangement.

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CORNELL UNIVERSITY,
ITHACA, NEW YORK.

Explanation of plates 18 and 19

All figures were drawn with the aid of an Abbé camera lucida.

FIG. 1. Nucellus with archesporial cell.

FIG. 2. Archesporial cell dividing.

FIG. 3. The primary parietal cell and the megaspore mother-cell.

FIG. 4. Two parietal cells and the megaspore mother-cell.

FIG. 5. The enlarged megaspore mother-cell.

FIG. 6. The megaspore mother-cell buried four cells deep by the increase of parietal tissue.

FIGS. 7 and 8. The dividing micropylar cell in the axial row of megaspore-cells.

FIG. 9. The functional megaspore, with the degenerating functionless megaspores.

FIG. 10. The division of the embryo-sac mother-cell.

FIG. 11. The two-nucleate stage of the embryo-sac.

FIG. 12. The simultaneous division of the two nuclei to form the four-nucleate sac.

FIG. 13. An ovule with a mature embryo-sac, after fusion of polars and disappearance of antipodals; showing the position in ovule and relative size of the sac.

FIG. 14. Entire embryo-sac, showing characteristic appearance and arrangement of sac-nuclei just after fertilization.

FIG. 15. Mature embryo-sac, showing egg-apparatus and endosperm-nucleus.

FIG. 16. An ovule, showing embryo-sac ready for fertilization.

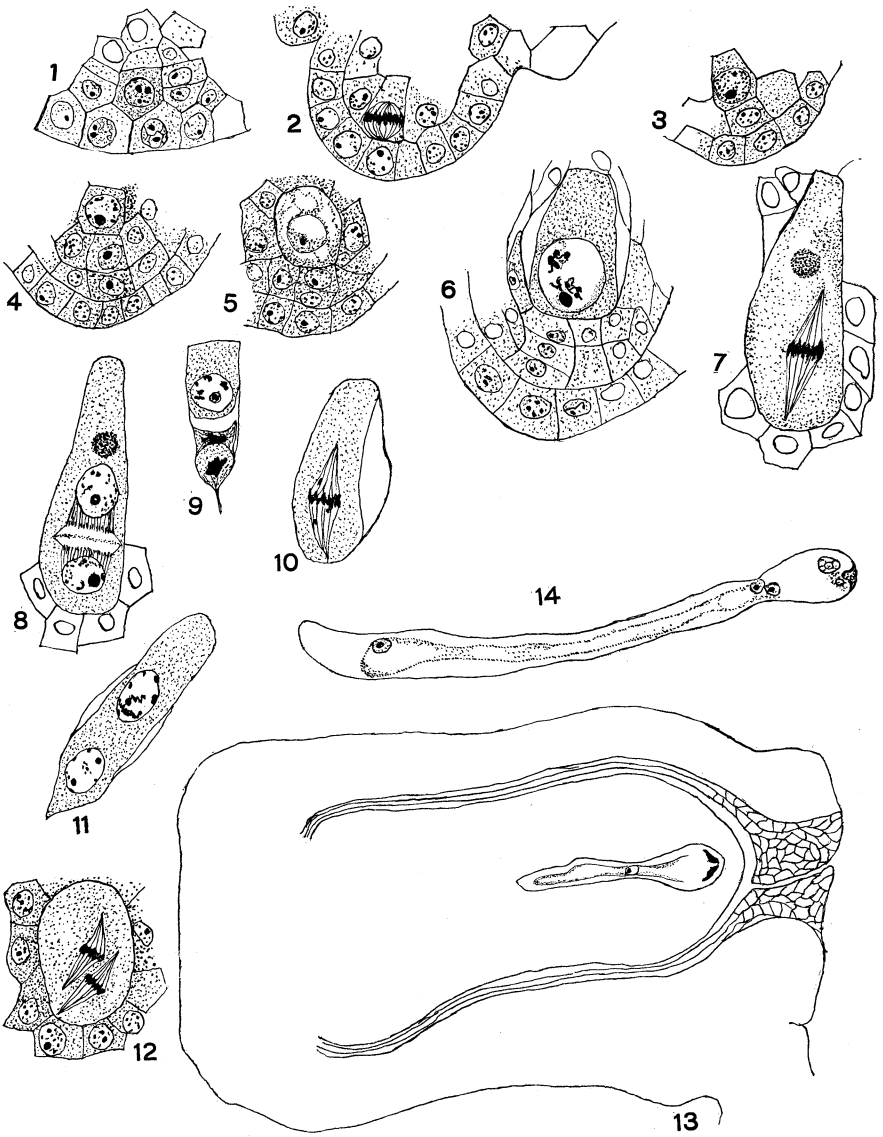
FIG. 17. Upper end of a sac, showing condensed cells between sac and the micropyle.

FIG. 18. The upper part of sac no. 14, showing details.

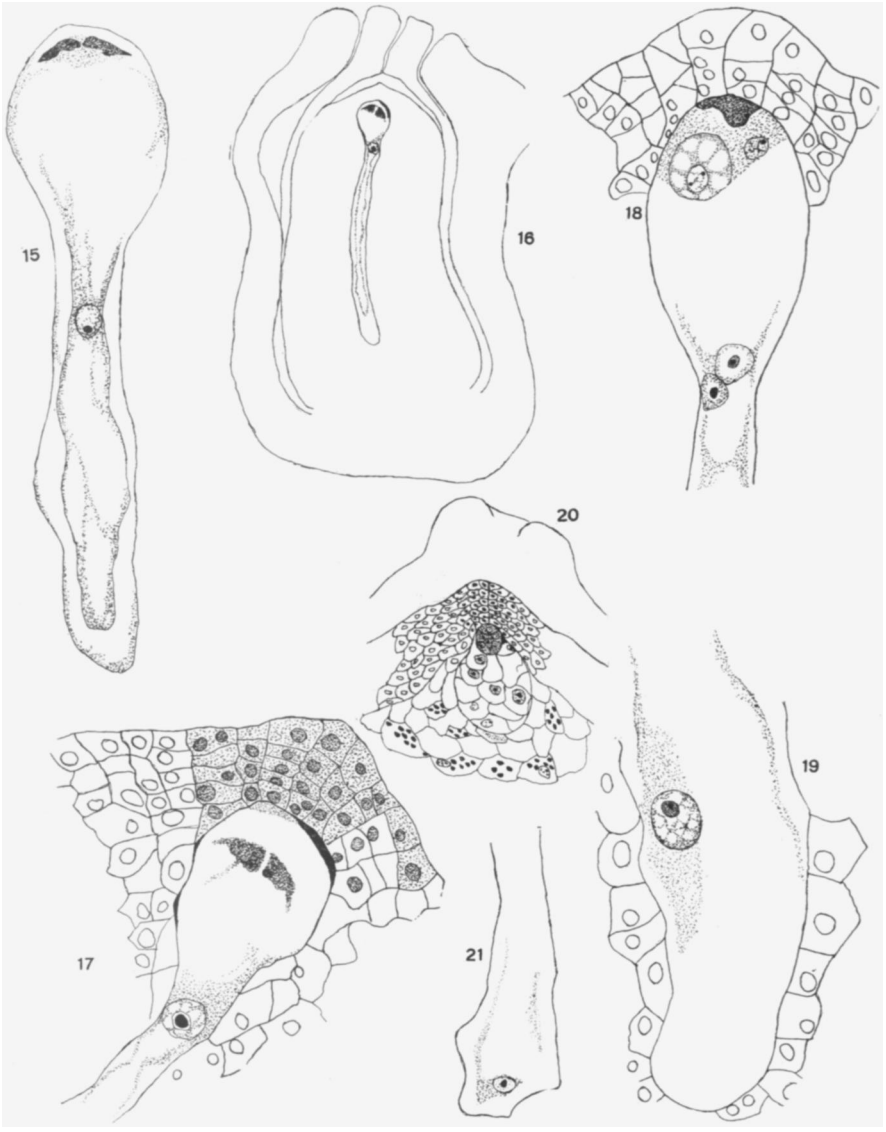
FIG. 19. The lower part of sac no. 14, showing details.

FIG. 20. The embryo with endosperm tissue and perisperm tissue containing starch.

FIG. 21. The chalazal end of ovule in 20, showing the persisting lower endosperm-nucleus.



SEATON, EMBRYO-SAC OF NYMPHAEA ADVENA



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